L-Selectin Shedding assay

General information

L-selectin shedding from the surface of leucocytes is a marker of activation. Toll-like receptors (TLRs) employ complex signalling pathways to generate an appropriate response to bacteria and viruses. A defect in these pathways can result in an impaired or absent immune response. This test is used to measure the amount of L-Selectin (CD62L) shed from the surface of neutrophils before and after stimulation of the TLR pathway. The test can help identify patients with defects in their toll-like receptor signalling pathway and can be used to diagnose IRAK-4, MyD88 and UNC93B deficiency.

Assay Interferences: Blood anticoagulated with EDTA preservative is not acceptable as it is acts as a calcium chelator (most cellular functions require calcium) therefore sub optimal results may be generated.
Quality of the sample received: Pre-activation of the samples can occur due to for example, infection or inadvertent activation caused by sample collection or transport to the lab with a concomitant reduction in CD62L expression.

Laboratory information

Units: % of neutrophils positive for CD62L expression

Specimen type: A minimum of 1ml blood in preservative-free heparin, with the sample <8hrs old. The department should be notified prior to venepuncture of the intention to request a L-Selectin shedding test (phone: 0161-276-6440 or extension 66440). In order to obtain a fresh uncompromised sample the whole blood for this assay must be received in a sterile universal container of preservative free heparin (10 units/mL). An age matched quality control sample from a normal donor should be supplied along with the patient's sample.

Frequency of analysis: Repeat only indicated if the test has failed (particularly if the control sample has failed).

Turnaround times (calendar days from sample receipt to authorised result): Mean - 2.

Specimen transport: At room temperature. Sample must be received <8hrs old and **before 12 noon** on weekdays only (not including bank holidays) to allow for processing and analysis.

Method: Flow cytometry

Participation in EQA Scheme: An EQA scheme is not available for this assay

Clinical Information

Reference range: A normal healthy patient sample should have >80% CD62L expression on the un-stimulated cells and shed <10% CD62L in response to all TLR agonists. The cell activator, Phorbol myristate acetate (PMA) is included as a positive control.

Interpretation: Patients with IRAK-4 or MyD88 deficiency will still express CD62L after treatment with lipopolysaccharide (LPS, TLR4 agonist) and the compound CL097 (TLR7/8 agonist) but have lost CD62L expression as a result of treatment with PMA. UNC-93B deficient patients will still express CD62L with CL097 but have lost CD62L with both LPS and PMA.

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